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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/27, 38/05</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/21459</b> <b>(43) International Publication Date:</b> 18 July 1996 (18.07.96)
<b>(21) International Application Number:</b> PCT/DK96/00016 <b>(22) International Filing Date:</b> 11 January 1996 (11.01.96) <b>(30) Priority Data:</b> 08/372,524 13 January 1995 (13.01.95) US <b>(71) Applicant:</b> NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). <b>(72) Inventors:</b> CHRISTENSEN, Thorkild; 55 Bellisvej, DK-3450 Allerød (DK). BALSCHMIDT, Per; 20 Tipperup Allé, DK-3060 Espergaerde (DK). SØRENSEN, Hans, Holmegaard; 21 Joachim Rønnowsvej, DK-2830 Virum (DK). OLSEN, Ole, Hvilsted; 38 Bækkeskovvej, DK-2700 Broenshoej (DK). THIM, Lars; 22 Skiftevej, DK-2820 Gentofte (DK).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  BEST AVAILABLE COPY
<b>(54) Title:</b> A STABILIZED PHARMACEUTICAL FORMULATION COMPRISING A GROWTH HORMONE AND LYS-X  <b>(57) Abstract</b>  A pharmaceutical formulation comprising a growth hormone and Lys-X, wherein X designates an amino acid residue, an additive or buffering substance, shows a very high stability against deamidation, oxidation and cleavage of peptide bonds. The stability of the product allows for the storing and shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved formulation at ambient temperature.		

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## TITLE

A stabilized pharmaceutical formulation comprising a growth hormone and Lys-X

## FIELD OF THE INVENTION

The present invention relates to a stabilized pharmaceutical formulation comprising  
5 growth hormone, to a method of making such formulation, and the use of Lys-X,  
wherein X designates an amino acid residue, for stabilizing a formulation of growth  
hormone.

## BACKGROUND OF THE INVENTION

The growth hormones from man and from the common domestic animals are  
10 proteins of approximately 191 amino acids, synthesized and secreted from the  
anterior lobe of the pituitary gland. Human growth hormone consists of 191 amino  
acids.

Growth hormone is a key hormone involved in the regulation of not only somatic  
growth, but also in the regulation of metabolism of proteins, carbohydrates and  
15 lipids. The major effect of growth hormone is to promote growth.

The organ systems affected by growth hormone include the skeleton, connective  
tissue, muscles, and viscera such as liver, intestine, and kidneys.

Until the development of the recombinant technology and the cloning of the growth  
hormone gene now giving rise to production of e.g. human growth hormone (hGH)  
20 and Met-hGH in industrial scale, human growth hormone could only be obtained by  
extraction from the pituitary glands of human cadavers. The very limited supplies of  
growth hormone restricted the use thereof to longitudinal growth promotion in  
childhood and puberty for treatment of dwarfism, even though it has been proposed  
for inter alia treatment of short stature (due to growth hormone deficiency, normal

short stature and Turner syndrome), growth hormone deficiency in adults, infertility, treatment of burns, wound healing, dystrophy, bone knitting, osteoporosis, diffuse gastric bleeding, and pseudoarthrosis.

Furthermore, growth hormone has been proposed for increasing the rate of growth of domestic animals or for decreasing the proportion of fat in animals to be slaughtered for human consumption.

Pharmaceutical formulations of growth hormone tend to be unstable. Degradation products such as deamidated or sulfoxidated products and dimer or polymer forms are generated - especially in solutions of growth hormone.

10 The predominant degradation reactions of hGH are 1) deamidation by direct hydrolysis or via a cyclic succinimide intermediate to form various amounts of L-asp-hGH, L-iso-asp-hGH, D-asp-hGH, and D-iso-asp-hGH (ref 1-3), and 2) oxidation of the methionine residues in positions 14 and 125 (ref 4-9). The major degradation product of hGH in lyophilized state as well as in solution is deamidated hGH.

15 Deamidation especially takes place at the Asn in position 149 and to a minor extent in position 152 and 99.

hGH is also rather easily oxidized in positions 14 and 125, especially in solution (4-8).

The oxidation of hGH in solution forming sulfoxides is normally due to the oxygen dissolved in the formulation. The solubility of oxygen in distilled water is about 200  $\mu\text{M}$  (9). As the concentration of hGH in a formulation comprising 4 IU/ml is 1.3 mg/ml corresponding to 60nM hGH, oxygen will, at normal storing conditions, be present in an excess of about 3000 times the stoichiometric amount for oxidation of hGH. It is not feasible to try to solve the problem by degassing of buffers before tapping and packing the formulations.

At present, it is not believed that these deamidated forms and oxidized forms of hGH should have toxic or altered biological activity or receptor binding properties, but there is indication to the effect that the conformation stability of the sulfoxides is reduced as compared to native hGH.

5 For the development of a stable, dissolved formulation comprising hGH it is of importance to know the rate of deamidation and formation of sulfoxides as well as means to control the reactions.

The kinetics of degradation depend on temperature, pH and various additives or adjuvants in the hGH formulation.

10 Due to the instability, growth hormone is, at present, lyophilized and stored in the lyophilized form at 4°C until it is reconstituted for use in order to minimize the degradation.

The lyophilized pharmaceutical formulations comprising hGH are, at present, reconstituted by the patient and then stored as a solution during the use for a period of  
15 up to 14 days at 4°C, during which some degradation will take place.

Furthermore, the process of reconstitution of the lyophilized growth hormone tends to provide difficulties for the patient.

Thus, it is at present preferred to reconstitute the growth hormone as late as possible before use and to store and ship the formulation in a lyophilized state. The  
20 chain from the manufacturer to the pharmacy is apt for handling the formulations at a controlled low temperature of e.g. 4°C which allows for a long shelf life of up to two years.

However, the extended use of pen systems for self-medication and the expanded field of use calls for a formulation which is stable for a sufficient long time with the  
25 end user under conditions where "sufficient" cooling is not always available.

Preferably, a formulation should be stable with the end user in a lyophilized state for about one month and additionally for one month in a reconstituted state in a pen device for the intended period of use of a cartridge.

Thus, there is a need for more stable formulations of growth hormone being stable  
5 in a lyophilized state at a relative high temperature for a period and additionally for a period of use at a relatively high temperature in solution. Such stabilization is of very great importance when moving the administration of the growth hormone from clinics to the homes of the individuals to be treated where optimal storage may not be available as indicated above.

10 Furthermore, the shift in pattern of administration of growth hormone to the use of pen devices calls for a stable dissolved formulation comprising growth hormone in order to facilitate the handling to be performed by the patient. A stable dissolved formulation comprising growth hormone may be produced ready to use in the form of cartridges fitting into the pen device used by the patient who may then avoid the  
15 reconstitution of the formulation and, hence, will not have to be in the possession of a lyophilized formulation, a suitable vehicle for reconstitution as well as the necessary skill and sterile equipment for sterile reconstitution of the formulation.

For safety reasons it will also be desirable to avoid the reconstitution of a lyophilized formulation just before the use of the formulation.

20 Furthermore, it would also be an advantage to avoid the lyophilization step in the production of growth hormone formulations. Lyophilization is a time consuming and costly process and is also often a "bottleneck" in the production due to the limited capacity of the freeze drier.

Thus, there is a need to reduce the rate of the degradation processes in order to  
25 allow for dissolved hGH formulations being stable during shelf life and during the period of use of up to one month.

Prior attempts to stabilize hGH has not fully succeeded in preventing the formation of dimer. The problems associated with dimer formation is e.g noted in Becker, G.W., Biotechnology and Applied Biochemistry 9, 478 (1987).

International Patent Publication No. WO 89/09614 and Australian patent application  
5 No. 30771/89 disclose a stable pharmaceutical formulation containing human growth hormone, glycine, and mannitol. Such a formulation shows improved stability during normal processing and storage in a lyophilized state as well as in the period of use after the reconstitution.

Published European patent application No. 303 746 discloses that animal growth  
10 hormone may be stabilized with various stabilizers to give decreased formation of insolubles and preservation of the soluble activity in aqueous environments, such stabilizers including certain polyols, amino acids, polymers of amino acids having a charged side group at physiological pH, and choline salts. Polyols are selected from the group consisting of non-reducing sugars, sugar alcohols, sugar acids,  
15 pentaerythritol, lactose, water-soluble dextrans and Ficoll; amino acids are selected from the group consisting of glycine, sarcosine, lysine or salts thereof, serine, arginine or salts thereof, betaine, N,N-dimethyl-glycine, aspartic acid or salts thereof, glutamic acid or salts thereof; a polymer of an amino acid having a charged side group at physiological pH may be selected from polylysine, polyaspartic acid,  
20 polyglutamic acid, polyarginine, polyhistidine, polyornithine and salts thereof; and choline derivatives are selected from the group consisting of choline chloride, choline dihydrogen citrate, choline bitartrate, choline bicarbonate, tricholine citrate, choline ascorbate, choline borate, choline gluconate, choline phosphate, di(choline)sulphate and dicholine mucate.

25 US patent specification No. 4,917,685 discloses a delivery device designed to be implanted comprising growth hormone stabilized using the same stabilizers as mentioned in EP 303746.

Published European patent application No. 374,120 discloses a stabilized formulation comprising hGH and a polyol having three hydroxy groups. Glycerol and tris(hydroxymethyl)aminomethane are mentioned. Furthermore, the presence of histidine hydrochloride as a buffer together with the polyol is disclosed.

5 International Patent Publication No. WO 93/12811 discloses stabilized formulations of growth hormone in the form of a lyophilized powder or an aqueous solution comprising asparagine.

International Patent Publication No. WO 93/12812 discloses stabilized formulations of growth hormone in the form of a lyophilized powder or an aqueous solution comprising histidine. In such formulations the deamidation is reduced by 25-30% as  
10 compared to a corresponding formulation of growth hormone comprising phosphate buffer.

International Patent Publication No. WO 93/19776 discloses protein formulations comprising growth hormone comprising citrate as buffer substance being more  
15 stable than formulations comprising phosphate buffer. The formulations may also comprise amino acids such as glycine and alanine and/or mannitol or other sugar alcohols and/or glycerol and/or other carbohydrates and optionally a preservative such as benzyl alcohol.

International Patent Publication No. WO 94/03198 discloses a stable aqueous  
20 formulation containing human growth hormone, a buffer, a non-ionic surfactant, and, optionally, a neutral salt, mannitol, or, a preservative.

## BRIEF DESCRIPTION OF THE INVENTION

It has now surprisingly been found that a formulation of human growth hormone comprising Lys-X, wherein X designates an amino acid residue show a very high  
25 stability against deamidation. The stability of the product allows for the storing and



shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved formulation.

The pharmaceutical formulations of the invention may be formulated for administration in any suitable way, e.g. by parenteral or oral administration or  
5 administration to a mucosal membrane, e.g. nasal administration. The pharmaceutical formulation may be presented in the form of a dose comprised in a vial or cartridge or any other suitable container such as a prefilled syringe or a pen device.

Thus, the formulation of the invention may be in the form of a lyophilized powder  
10 to be reconstituted later using conventional vehicles such as distilled water or water for injection or in the form of a solution comprising growth hormone. Such vehicles may comprise conventional preservatives such as m-cresol and benzyl alcohol.

X designates preferably a naturally occurring amino acid residue, more preferred a naturally occurring  $\alpha$ -amino acid residue, and most preferred Ala, Gly, Leu, Lys or  
15 Asp.

A preferred embodiment of the invention is in the form of a pharmaceutical formulation of human growth hormone comprising Lys-X, wherein X designates an amino acid residue and further comprising a carrier in the form of a buffered aqueous solution of growth hormone. Such formulation is in a ready-to-use form and  
20 may be stored and shipped as an aqueous solution without any considerable degradation.

A buffer to be used in a solution of growth hormone may e.g. be histidine, citrate, tartrate or phosphate buffer.

For stability reasons the pH of a solution is preferably adjusted to a value in the  
25 interval from about 2 to about 8, more preferred to pH from 5 to 7, especially to about 6.8.

In order to obtain the stabilizing effect Lys-X, wherein X designates an amino acid residue is preferably added in an amount of up to 100 mM, more preferred in an amount of about 1-10 mM, preferably 2-6 mM, most preferred 3-5 mM.

The pharmaceutical formulation of the invention may furthermore comprise salts for  
5 adjusting the tonicity and optionally an excipient in order to facilitate the processing thereof, e.g. lyophilization and the rapid and complete dissolution of a lyophilized formulation when reconstituting the formulation before use.

An excipient may be selected from disaccharides such as lactose, trehalose, and sucrose, sugar alcohols such as sorbitol or mannitol, polysaccharides such as the  
10 polymers commercialized as Dextran® products such as Dextran® 40, Dextran® 70 or Dextran® 75, and Ficoll® and polyvalent alcohols such as polyethylene glycol or polyvinyl alcohol or a combination of two or more of these.

In a further aspect the invention relates to a method of preparing a pharmaceutical formulation comprising a growth hormone and Lys-X, wherein X has the meaning  
15 stated above, wherein the growth hormone is dissolved in a solution comprising Lys-X, wherein X has the meaning stated above, by dissolving Lys-X, wherein X has the meaning stated above, in deionized water optionally containing of benzyl alcohol, adding the growth hormone and optionally adjusting the pH to from about 2 to about 8.

20 The pH may be adjusted by adding an acid which has no adverse effect on the growth hormone, preferably a physiologically acceptable acid e.g. a mineral acid such as hydrochloric acid, sulphuric acid or nitric acid or an organic acid such as acetic acid.

In an embodiment of the method of the invention, is added optionally salts and an  
25 excipient, whereafter the solution is filled into a container and lyophilized.

Still another aspect of the invention relates to the use of Lys-X, wherein X designates an amino acid residue, for the formulation of a stabilized formulation of growth hormone.

In the present context "growth hormone" may be growth hormone from any origin  
5 such as avian, bovine, equine, human, ovine, porcine, salmon, trout or tuna growth hormone, preferably bovine, human or porcine growth hormone, human growth hormone being most preferred. The growth hormone used in accordance with the invention may be native growth hormone isolated from a natural source, e.g. by extracting pituitary glands in a conventional manner, or a growth hormone produced  
10 by recombinant techniques, e.g. as described in E.B. Jensen and S. Carlsen in Biotech and Bioeng. 36, 1-11 (1990). The "growth hormone" may also be a truncated form of growth hormone wherein one or more amino acid residues has (have) been deleted; an analogue thereof wherein one or more amino acid residues in the native molecule has (have) been substituted by another amino acid residue, preferably a  
15 natural amino acid residue, as long as the substitution does not have any adverse effect such as antigenicity or reduced action; or a derivative thereof, e.g. having an N- or C-terminal extension such as Met-hGH. The preferred growth hormone is hGH.

The term "dose" of growth hormone refers to that amount that provides therapeutic  
20 effect in an administration regimen. The formulations hereof are prepared containing amounts of hGH at least about 0.1 mg/ml, preferably upwards of about 10 mg/ml, preferably from about 1 mg/ml to about 40 mg/ml, more preferably from about 1 mg/ml to about 25 mg/ml, e.g. from 1 mg/ml to about 5 mg/ml, calculated on the ready-to-use formulation. For use of these compositions in administration to  
25 human beings suffering from hypopituitary dwarfism, for example, these formulations contain from about 0.1 mg/ml to about 10 mg/ml, corresponding to the currently contemplated dosage regimen for the intended treatment. The concentration range is not critical to the invention and may be varied by the physician supervising the administration.

Lys-X, wherein X designates an amino acid residue to be used in accordance with the present invention is preferably comprising the naturally occurring alpha amino acid residues. The amino acid(s) may be l or d amino acid(s) or a mixture thereof.

In the present context "high stability" is obtained when the formulation is more  
5 stable than the conventional formulation comprising phosphate buffer and preferably as stable as a corresponding formulation comprising histidine as stabilizer in which the de-amidation of hGH is reduced by approximately 20% as compared with phosphate buffer as disclosed in WO 93/12812.

The solvent used in the method of the invention may be water, alcohols such as  
10 ethyl, n-propyl or isopropyl, butyl alcohol or mixtures thereof. The solvent may comprise a preservative such as m-cresol or benzyl alcohol.

## DETAILED DESCRIPTION OF THE INVENTION

The invention is explained more in detail in the below Examples which illustrate the invention. They are not to be considered as limiting the scope of the invention being defined by the appended claims.

### 5 EXPERIMENTAL PART

#### EXAMPLE

##### Reduction of the deamidation.

The rate of deamidation was examined at 37°C for hGH formulations comprising 4 mg/ml hGH at pH 6.8 in the presence of 5 mM Lys-Ala, Lys-Gly, Lys-Leu, Lys-Lys  
10 or Lys-Asp as compared to histidine at pH 6.8.

The hGH formulations were prepared by dissolving 8 mg hGH in 2 ml of 10 mM solution of the dipeptide or histidine. Thus, 2 ml 3.0% of benzyl alcohol was added to give a final formulation of 4 mg/ml hGH, 5 mM dipeptide or histidine, 1.5% benzyl alcohol, pH 6.8 (adjusted adding HCl or NaOH).

15 The hGH formulations stated in the below table were stored at 37°C for 7 days, and analysed for the content of deamidated hGH by IE-HPLC. The results appear from the below table.

Table

Formulation	pH start/pH end/% desamido	Corrected content of deamidated hGH*	Contents of des-amido hGH as compared with His
Lys-Ala	6.8/6.8/15.8	15.8	92
Lys-Gly	6.8/6.9/16.1	15.6	91
5 Lys-Leu	6.8/6.9/16.6	16.1	94
Lys-Lys (4.7mM)	6.8/7.0/18.1	17.1	99
Lys-Asp	6.8/7.1/17.3	15.8	92
Histidine	6.8/7.2/19.2	17.2	100

\* Desamido corrected by 1% per 0.1 pH unit deviation from 6.8

10 The contents of deamidated hGH in the starting material was: 2.0%

From the above table it appears that the deamidation of hGH is reduced by addition of Lys-Ala, Lys-Gly, Lys-Leu, Lys-Lys or Lys-Asp to at least the same level as obtained by addition of histidine (25-30% as compared to phosphate buffer, cf. WO 93/12812 above).

15 The above results show that the rate of deamidation is reduced to a very great extent by adding Lys-Ala, Lys-Gly, Lys-Leu, Lys-Lys or Lys-Asp in a low concentration of up to 100 mM, preferably 1-10 mM, more preferred 2-6 mM and most preferred about 3-5 mM. The rate of deamidation may thus be reduced by more than 30% by substituting the phosphate buffer with Lys-Ala, Lys-Gly, Lys-Leu, Lys-Lys or  
20 Lys-Asp.

The use of benzyl alcohol as preservative seems to have no influence on the rate of deamidation.

## REFERENCES

- 1) Y.-C.J. Wang and M.A. Hanson. Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers. J. Parenteral Science and Technology 42 (Suppl.) (1988) 53-525.
- 5 2) M.C. Manning, K. Patel, R.T. Borchardt. Stability of Protein Pharmaceuticals. Pharmaceutical Research 6 (11) (1989) 903-918.
- 3) B.A. Johnson, J.M. Shirokawa, W.S. Hancock, M.W. Spellman, L.J. Basa and D.W. Asward. J.Biol.Chem. 264, 1462-71 (1989).
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- 10 5) G.W. Becker et al., Biotech.Appl.Biochem., 10, 326-337 (1988).
- 6) R.A. Houghten et al., Arch.Biochem.Biophys., 178, 350-355 (1977).
- 7) R.M. Riggin et al., Anal.Biochem., 167, 199-209 (1987).
- 8) P. Gellerfors et al., Acta Paediatr.Scand (suppl), 370, 93-100 (1990).
- 9) M.J. Kaufman, Pharm.Res., 7 (3) 289-292 (1990).

**CLAIMS**

1. A pharmaceutical formulation comprising a growth hormone and Lys-X, wherein X designates an amino acid residue.
2. A pharmaceutical formulation as claimed in claim 1 further comprising a carrier  
5 in the form of a buffered aqueous solution of growth hormone containing Lys-X, wherein X designates an amino acid residue.
3. A pharmaceutical formulation as claimed in claim 1 or 2 wherein the pH is adjusted to a value in the interval from about 2 to about 8.
4. A pharmaceutical formulation as claimed in any of the preceding claims wherein  
10 the concentration of Lys-X, wherein X designates an amino acid residue, is up to about 100 mM.
5. A pharmaceutical formulation as claimed in any of the preceding claims further comprising salts and saccharides.
6. A pharmaceutical formulation as claimed in any of the preceding claims wherein  
15 the growth hormone is hGH.
7. A method of preparing the pharmaceutical formulation of claim 1 comprising adding growth hormone to a solution comprising Lys-X.
8. A method for stabilizing a formulation of growth hormone comprising adding an amount of Lys-X effective to stabilize said formulation.
- 20 9. The method according to claim 7 or 8, in which the solution comprising Lys-X is obtained by dissolving Lys-X in deionized water.



10. The method according to any of the preceding claims, in which the solution comprising Lys-X also comprises benzyl alcohol.
11. The method according to any of the preceding claims, which further comprises adjusting the pH value of said formulation to from about 2 to about 8.
- 5 12. The method according to any of the preceding claims, which further comprises adding salts.
13. The method according to any of the preceding claims, which further comprises adding an excipient.
14. The method according to any of the preceding claims, which further comprises  
10 filling a solution comprising growth hormone and Lys-X into a container and lyophilizing said solution.
15. Use of Lys-X , wherein X designates an amino acid residue, for the formulation of a stabilized formulation of growth hormone.
16. A method for treating a disorder in a patient affectable by growth hormone,  
15 comprising treating the patient with an amount of the pharmaceutical formulation of claim 1-6 effective to treat said disorder.
17. Any novel feature or combination of features disclosed herein.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00016

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/27, A61K 38/05

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, MEDLINE, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9312812 A1 (NOVO NORDISK A/S), 8 July 1993 (08.07.93), page 15, line 16 - line 23 --	1-15
A	WO 9118621 A1 (GENENTECH, INC.), 12 December 1991 (12.12.91), page 10, line 20 - line 28 --	1-15
A	US 4816568 A (EDWIN J. HAMILTON, JR. ET AL), 28 March 1989 (28.03.89) --	1-15
A	WO 9312811 A1 (NOVO NORDISK A/S), 8 July 1993 (08.07.93) -- -----	1-15

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

25 April 1996

Date of mailing of the international search report

29 -04- 1996

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00016

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
See PCT Rule 39.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☒ Claims Nos.: 17  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The claim does not define the matter for which protection is sought.  
It is not clear and concise, see Art 6.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.

01/04/96

PCT/DK 96/00016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9312812	08/07/93	AU-A- 3344693	28/07/93
		BG-A- 98806	28/02/95
		CA-A- 2125855	08/07/93
		CN-A- 1096222	14/12/94
		CZ-A- 9401507	18/01/95
		EP-A- 0618807	12/10/94
		FI-A,D- 942906	17/06/94
		HU-A- 69402	28/09/95
		HU-D- 9401832	00/00/00
		JP-T- 7502516	16/03/95
		NO-A,D- 942300	19/08/94
		NZ-A- 246556	26/03/96
		SK-A- 75494	08/03/95
		ZA-A- 9209825	23/06/93
WO-A1- 9118621	12/12/91	EP-A,A- 0536226	14/04/93
		US-A- 5126324	30/06/92
		US-A- 5374620	20/12/94
US-A- 4816568	28/03/89	NONE	
WO-A1- 9312811	08/07/93	AU-B- 664416	16/11/95
		AU-A- 3344593	28/07/93
		CA-A- 2125856	08/07/93
		CZ-A- 9401458	15/12/94
		EP-A- 0618806	12/10/94
		FI-A,D- 942905	17/06/94
		HU-A- 69682	28/09/95
		HU-D- 9401831	00/00/00
		JP-T- 7502515	16/03/95
		NO-A,D- 942299	17/06/94
		NZ-A- 246555	26/03/96
		SK-A- 73794	08/03/95

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